

In the claims:

1. (Currently amended) A method of identifying interactions between polypeptides comprising:

(a) ~~expressing~~ ^{superimposed} expressing in a cell lacking Ras activity:

(i) a first polynucleotide encoding a first polypeptide being capable of interacting with a plasmalemma of the cell and being operably linked to an inducible promoter; and

(ii) a second polynucleotide encoding a second polypeptide fused to a cytoplasmic Ras mutant, said cytoplasmic Ras mutant being capable of said Ras activity if mobilized to said plasmalemma of said cell; and

(b) detecting Ras activity in said cell grown under:

(i) inductive conditions which result in expression of said first polypeptide from said inducible promoter; and

(ii) suppressive conditions which result in substantially no expression of said first polypeptide from said inducible promoter,

wherein said Ras activity present only in said cell grown under said inductive conditions is indicative of an interaction between said first polypeptide and said second polypeptide.

2. (Original) The method of claim 1, wherein said first polypeptide is a native membrane protein.

3. (Cancelled) The method of claim 1, further comprising:

(c) independently expressing in said cell substantially only said second polynucleotide to thereby determine if said Ras activity detected in step (b) is dependent on said interaction between said first polypeptide and said second polypeptide.

in orig claim
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claim 6

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4. (Cancelled) The method of claim 3, wherein said first polypeptide is a native membrane protein.

5. (Cancelled) The method of claim 3, wherein an expression of said first polynucleotide and/or said second polynucleotide in said cell is separately regulatable such that step (c) is effected by downregulating an expression of said first polynucleotide and/or upregulating an expression of said second polynucleotide.

6. (Previously presented) The method of claim 1, wherein said cell lacking Ras activity is a yeast cell exhibiting a mutant Ras phenotype characterized by growth suppression under non-permissive conditions.

7. (Original) The method of claim 6, wherein said cytoplasmic Ras mutant is capable of complementing said mutant Ras phenotype if mobilized to said plasmalemma of said cell.

8. (Original) The method of claim 1, wherein said first polypeptide includes an amino acid sequence for plasmalemma targeting.

9. (Currently amended) A method of identifying interactions between polypeptides comprising:

(a) ¹ expressing in cells lacking a Ras activity:

(i) a first polynucleotide encoding a first polypeptide being capable of interacting with a plasmalemma of said cells and operably linked to an inducible promoter; and

(ii) a library of polynucleotides each encoding a distinct polypeptide ~~fused~~ to a cytoplasmic Ras mutant, said cytoplasmic Ras mutant being capable of said Ras activity if mobilized to said plasmalemma of said cells; and

(b) identifying said Ras activity in said cells grown under:

(i) inductive conditions which result in expression of said first polypeptide from said inducible promoter; and

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- (ii) suppressive conditions which result in substantially no expression of said first polypeptide from said inducible promoter,

wherein said Ras activity present only in said cells grown under said inductive conditions is indicative of an interaction between said first polypeptide and said distinct polypeptide.

10. (Previously presented) The method of claim 9, further comprising isolating from each cell of said cells a polynucleotide encoding said distinct polypeptide.

11. (Original) The method of claim 9, wherein said first polypeptide is a native membrane protein.

12. (Cancelled) The method of claim 9, further comprising:

- (c) independently expressing in said cells substantially only said library of polynucleotides to thereby determine if said Ras activity exhibited in said subset of cells is dependent on an interaction between said first polypeptide and said distinct polypeptide.

13. (Cancelled) The method of claim 12, wherein said first polypeptide is a native membrane protein.

14. (Cancelled) The method of claim 12, wherein an expression of said first polynucleotide and/or said library of polynucleotides in said cells is separately regulatable such that step (c) is effected by downregulating an expression of said first polynucleotide and/or upregulating an expression of said library of polynucleotides.

15. (Previously presented) The method of claim 9, wherein said cells lacking said Ras activity are yeast cells exhibiting a mutant Ras phenotype characterized by growth suppression under non-permissive conditions.

16. (Original) The method of claim 15, wherein said cytoplasmic Ras mutant is capable of complementing said mutant Ras phenotype if mobilized to said plasmalemma of said cells.

17. (Original) The method of claim 9, wherein said first polypeptide includes an amino acid sequence for plasmalemma targeting.

18. (Currently amended) A method of identifying interactions between polypeptides comprising:

(a) ~~expressing~~ expressing in cells lacking a Ras activity:

- (i) a library of polynucleotides each encoding a first polypeptide being capable of interacting with a plasmalemma of said cells fused to a second polypeptide; and
- (ii) a second polynucleotide encoding a cytoplasmic Ras mutant fused to a third polypeptide and and being operably linked to an inducible promoter, said cytoplasmic Ras mutant being capable of said Ras activity if mobilized to said plasmalemma of said cells; and

(b) identifying Ras activity in said cells grown under:

- (i) inductive conditions which result in expression of said first polypeptide from said inducible promoter; and
- (ii) suppressive conditions which result in substantially no expression of said first polypeptide from said inducible promoter,

wherein said Ras activity present only in said cells grown under said inductive conditions is indicative of an interaction between said third polypeptide and said second polypeptide.

19. (Previously presented) The method of claim 18, further comprising isolating from each cell of said cells a polynucleotide encoding said second polypeptide.

20. (Original) The method of claim 18, wherein said first polypeptide is a native membrane protein.

21. (Cancelled) The method of claim 18, further comprising:

(c) independently expressing in said cells substantially only said second polynucleotide to thereby determine if said Ras activity exhibited in said subset of cells is dependent on an interaction between said third polypeptide and said second polypeptide.

22. (Cancelled) The method of claim 21, wherein said first polypeptide is a native membrane protein.

23. (Cancelled) The method of claim 21, wherein an expression of said library of polynucleotides and/or said second polynucleotide in said cells is separately regulatable such that step (c) is effected by downregulating an expression of said library of polynucleotides and/or upregulating an expression of said second polynucleotide.

24. (Previously presented) The method of claim 18, wherein said cells lacking said Ras activity are yeast cells exhibiting a mutant Ras phenotype characterized by growth suppression under non-permissive conditions.

25. (Original) The method of claim 24, wherein said cytoplasmic Ras mutant is capable of complementing said mutant Ras phenotype if mobilized to said plasmalemma of said cells.

26. (Original) The method of claim 18, wherein said first polypeptide includes an amino acid sequence for plasmalemma targeting.

27. (Currently amended) A method of identifying interactions between polypeptides comprising:

(a) expressing in cells lacking a Ras activity:

- (i) a first library of polynucleotides each operably linked to an inducible promoter and encoding a first polypeptide being capable of interacting with a plasmalemma of said cells fused to a second polypeptide; and
- (ii) a second library of polynucleotides each encoding a cytoplasmic Ras mutant fused to a third polypeptide, said cytoplasmic Ras mutant being capable of said Ras activity if mobilized to said plasmalemma of said cells; and

(b) identifying said Ras activity in said cells grown under:

- (i) ~~inductive conditions which result in expression of said first polypeptide from said inducible promoter; and~~
- (ii) ~~suppressive conditions which result in substantially no expression of said first polypeptide from said inducible promoter;~~

~~wherein said Ras activity present only in said cells grown under said inductive conditions is indicative of an interaction between said third polypeptide and said second polypeptide.~~

28. (Previously presented) The method of claim 27, further comprising isolating from each cell of said cells polynucleotides encoding said second polypeptide and said third polynucleotides.

29. (Original) The method of claim 27, wherein said first polypeptide is a native membrane protein.

30. ~~(Cancelled) The method of claim 27, further comprising:~~

- ~~(c) independently expressing in said cells substantially only said second library of polynucleotides to thereby determine if said Ras activity exhibited in said subset of cells is dependent on an interaction between said third polypeptide and said second polypeptide.~~

31. ~~(Cancelled) The method of claim 30, wherein said first polypeptide is a native membrane protein.~~

32. (Cancelled) The method of claim 30, wherein an expression of said first library of polynucleotides and/or said second library of polynucleotides in said cells is separately regulatable such that step (c) is effected by downregulating an expression of said first library of polynucleotides and/or upregulating an expression of said second library of polynucleotides.

33. (Previously presented) The method of claim 27, wherein said cells lacking said Ras activity are yeast cells exhibiting a mutant Ras phenotype characterized by growth suppression under non-permissive conditions.

34. (Original) The method of claim 33, wherein said cytoplasmic Ras mutant is capable of complementing said mutant Ras phenotype if mobilized to said plasmalemma of said cells.

35. (Original) The method of claim 27, wherein said first polypeptide includes an amino acid sequence for plasmalemma targeting.

36. (Withdrawn) A nucleic acid construct comprising a polynucleotide encoding a cytoplasmic Ras mutant under the transcriptional control of an inducible promoter.

37. (Withdrawn) The nucleic acid construct of claim 36, wherein said inducible promoter is functional in eukaryotic cells.

38. (Withdrawn) The nucleic acid construct of claim 36, wherein said inducible promoter is selected from the group consisting of a Gal1 promoter, a Yes2 promoter, a Met425 promoter and a copper inducible promoter.

39. (Withdrawn) A nucleic acid construct library comprising a plurality of nucleic acid constructs each including a first polynucleotide region encoding a

cytoplasmic Ras mutant translationally fused to a second polynucleotide region encoding a distinct polypeptide.

40. (Withdrawn) The nucleic acid construct library of claim 39, wherein each of said plurality of nucleic acid constructs further includes a promoter for directing the expression of said first and said second polynucleotide regions.

41. (Withdrawn) The nucleic acid construct library of claim 40, wherein said promoter is functional in eukaryotic cells.

42. (Withdrawn) The nucleic acid construct library of claim 40, wherein said promoter is an inducible promoter selected from the group consisting of a Gal1 promoter, a Yes2 promoter, a Met425 promoter and a copper inducible promoter.

43. (Withdrawn) A nucleic acid construct system comprising:
- (a) a first nucleic acid construct including a first polynucleotide encoding a first polypeptide being capable of interacting with a plasmalemma of a cell in which it is expressed; and
 - (b) a second nucleic acid construct including a second polynucleotide encoding a cytoplasmic Ras mutant fused to a second polypeptide.

44. (Withdrawn) The nucleic acid construct system of claim 43, wherein said first and said second nucleic acid construct each further include a promoter sequence for directing an expression of said first and said second polynucleotides in a eukaryotic cell.

45. (Withdrawn) The nucleic acid construct system of claim 44, wherein said first nucleic acid construct includes a first promoter sequence and said second nucleic acid construct includes a second promoter sequence, whereas said first and said second promoter sequences are independently selected from the group consisting of a constitutive promoter sequence and an inducible promoter sequence.

46. (Withdrawn) The nucleic acid construct system of claim 45, wherein said inducible promoter sequence is selected from the group consisting of a Gal1 promoter, a Yes2 promoter, a Met425 promoter and a copper inducible promoter.

47. (Withdrawn) A method of identifying polypeptides of interest comprising:

- (a) expressing in a cell substantially lacking Ras activity:
 - (i) a first polynucleotide encoding a first polypeptide being capable of modifying a plasmalemma of the cell; and
 - (ii) a second polynucleotide encoding a second polypeptide fused to a cytoplasmic Ras mutant, said cytoplasmic Ras mutant being capable of said Ras activity if mobilized to said plasmalemma of said cell; and
- (b) detecting a presence or absence of said Ras activity in said cell, said Ras activity being indicative of membrane mobilization of said second polypeptide resultant from a modification to said plasmalemma effected by said first polypeptide.

48. (Withdrawn) The method of claim 47, wherein said modifying said plasmalemma of the cell is effected by modifying a protein or phospholipid component of said plasmalemma.

49. (Withdrawn) The method of claim 47, wherein said first polypeptide is PI3kinase.